Docket No.: 000166.0073-US02
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
J. Michael Ramstack et al.

Application No.: 10/681,142 | Confirmation No.: 6453

Filed: October 9, 2003 | Art Unit: 1615

For: PREPARATION OF INJECTABLE SUSPENSIONS HAVING IMPROVED INJECTABILITY | Examiner: S. T. Tran

DECLARATION OF STEPHEN E. ZALE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Stephen E. Zale , declare that the following facts are true:

1. I am a co-inventor of the subject matter of the above-captioned application, which is assigned to Alkermes Controlled Therapeutics, Inc., a subsidiary of Alkermes, Inc. ("Alkermes"). I am not currently an employee of Alkermes, but I was an employee of Alkermes during the development of the subject matter of the above-captioned application.
2. Attached as Exhibit A is a copy of notebook pages that describe a human tissue injectability test that was carried out in the United States. All dates on the notebook pages have been removed from the copy attached as Exhibit A. However, the original notebook pages of Exhibit A bear dates that are prior to May 27, 1999.

3. Exhibit A describes a two step reconstitution experiment whereby microparticles were first mixed with an injection vehicle to suspend the microparticles, and then a viscosity enhancing agent was added to increase the viscosity. As described on notebook page 1 of Exhibit A, a 3% CMC (sodium carboxymethyl cellulose) injection vehicle with a viscosity of approximately 53 cp was used to suspend blank (placebo) microspheres (microparticles) having a polymeric binder. Addition of a 10% CMC vehicle was done to increase the viscosity to approximately 730 cp. A total volume of 1 ml of the microsphere suspension was then injected through a 22 gauge needle. There were no failures of any injection.
4. Attached as Exhibit B is a copy of the results of a study carried out in the United States to investigate the effects of viscosity on microsphere injectability. All dates on the pages have been removed from the copy attached as Exhibit B. However, the original pages of Exhibit B bear dates that are prior to May 27, 1999.
5. As described on pages 1 and 2 of Exhibit B, blank (placebo) microspheres (microparticles) were suspended in an aliquot of a 3% CMC injection vehicle. The microsphere suspension was then mixed in a 3 cc syringe with increasing amounts of 10% CMC injection vehicle. The viscosity of the fluid phase of the suspension ranged from about 53 cp to greater than 1000 cp, depending upon the ratio of the 3% and 10% injection vehicles. Injectability of the suspensions was evaluated using thawed porcine skin and a 22 gauge needle. Three replicates were run for each viscosity with each injection rated as a success (+) or failure (-).
6. Attached as Exhibit C is a copy of a draft report of a study carried out in the United States to determine the effect of particle size, injection vehicle viscosity and injection site on the injectability of risperidone microspheres in pigs. All dates on the pages have been

removed from the copy attached as Exhibit C. However, the original pages of Exhibit C bear dates that are prior to May 27, 1999.

7. Tables 2 and 3 of Exhibit C demonstrate that a "high" vehicle viscosity resulted in a low injection failure rate, and a "low" vehicle viscosity resulted in a high injection failure rate. The "high" vehicle viscosity of Tables 2 and 3 is approximately 27 cp at 20 °C. The "low" vehicle viscosity of Tables 2 and 3 is approximately 7 cp at 20 °C. The results reported in tables 2 and 3 of Exhibit C are reflected in Tables 2 and 3 of the above-captioned application.
8. Attached as Exhibit D is a copy of a Development Report on the effect of vehicle viscosity on injectability of RISPERDAL® depot (microparticles having a poly(d,l-lactide-co-glycolide) polymeric binder and the active agent risperidone). The report provides the results of a sheep study that was conducted in the United States. All dates on the pages have been removed from the copy attached as Exhibit D. However, the original pages of Exhibit D bear dates that are prior to May 27, 1999.
9. Tables 2 and 3 of Exhibit D again demonstrate that a higher vehicle viscosity resulted in fewer injection failures, and that an injection vehicle viscosity of at least about 20 cp is necessary for successful and medically acceptable injectability rates. The experiments were conducted using high suspension concentration (greater than 100 mg/ml), and a small needle gauge size (22 gauge). At viscosities of less than or equal to about 11 cp, injectability failures increase significantly. The results reported in tables 2 and 3 of Exhibit D are reflected in Tables 4 and 5 of the above-captioned application.

10. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false or misleading statements so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned patent application or any patent issued thereon.

Date: 24 May 06

Stephen E. Zale

INSTRUCTIONS

1. To secure adequate Patent-Rights is the primary purpose of this book. Only properly kept records will assure this Company of such protection. When starting a project, write in precise language your purpose, and general plan or procedure. Record your work as you progress, giving sufficient details. Handwrite directly in the book. Do not make notes on loose paper to be copied later.

2. All entries should be in ink. *Do not use pencil.*

3. The Title, project number, and book number should be accurately recorded when starting work.

4. In chronological order give a complete, accurate account of what you did, and what resulted. Enter all results, both good and bad. In case of error, draw a line through the incorrect words. Then continue with the correct wording. Copious descriptions with elaborate details are preferable. Better too much, than too little. Always keep in mind the necessity of original data to prove any new discoveries.

5. Complete calculations in detail should be written in this book and becomes our proprietary property.

6. Names of Operators, and Witnesses who are present during the demonstration should be recorded. At least one witness, not claiming to be a co-discoverer should sign and date in the space provided at bottom of work sheet. New concepts, and new solutions to problems should be witnessed by your co-workers, or someone competent to understand the language and materials being recorded. These facts should be recorded, signed and dated.

7. New ideas, plans, procedures, sketches, etc., should be recorded immediately in this book at the time they occur. These should be *disclosed to, and understood by* your co-workers, who sign and date this fact.

8. When an experiment shows results of possible patentable importance, and No Witnesses are present, the procedure should be repeated under you supervision by your co-worker as soon as possible. Data covering the experiment should be recorded in both yours, and his Notebooks, with proper signatures and dates.

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10. In general, only one subject should be recorded on each page. Long term projects should have separate books. All projects should be so recorded that any co-worker may continue the operation in your absence or re-assignment.

11. Pages are provided for a Table of Contents. This should be completed to enable ready access to the contents in the future.

Norman Kim -51

Assigned To DARRELL NIX Date Notebook No.

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1. Human tissue injectability	1

Human tissue Injectability

Objective: Determine how vehicle viscosity effects microsphere sieving following injection (SC)

Blank Microspheres were used, at an injection concentration of 200 mg/1 ml.

Freshly prepared 3% CMC vehicle was used to suspend the microspheres. Addition of 10% CMC vehicle was done to increase viscosity to approximately 730 cP

A total volume of 1 ml of microsphere suspension was injected through a 22Ga TW needle.

Human tissue: NE-11-035 & NE-11-041

Photographs of the Quadrocept injection sites were taken using the Kodak Image Analyzer and using a 35mm camera duplicate photographs were also taken.

There were no failures of any injection. Following a series of injections the sites were cut open to observe the behavior of the injected suspension. In previous experiments it was observed that post injection sieving of the vehicle from the microspheres was occuring. This appeared to be an immediate event, resulting in separation of the microspheres mass from vehicle.

In order to reduce any effects that time might

SIGNATURE Russell Ng DATE

DISCLOSED TO AND UNDERSTOOD BY Douglas Bissonnette DATE WITNESS DATE

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have on the extent of dicing two suspensions were prepared nearly simultaneously to allow injections to be made one right after another.

The first injection was the high viscosity suspension followed immediately by a lower viscosity suspension.

There appears to be a slight difference in the behavior of the two suspensions. In the lower viscosity injection site vehicle tended to separate or sieve out away from the microsphere polymer material, leaving a slightly more dense white clump. In the high viscosity injection site the microsphere suspension remained more evenly dispersed showing little evidence of separation of vehicle from the microsphere polymer.

<u>NOTE</u>: 0.2 ml of green food coloring was added to 10 ml of 3% CMC vehicle to allow better visualization of the contrast between vehicle and microsphere polymer material. The food coloring doesn't turn of polymer green, it remains a white color.

A section of skin and fat layer was taken from each tissue sample that was injected and placed in 10% formalin in a heat sealed bag. These sections will be stained and processed for histopathology.

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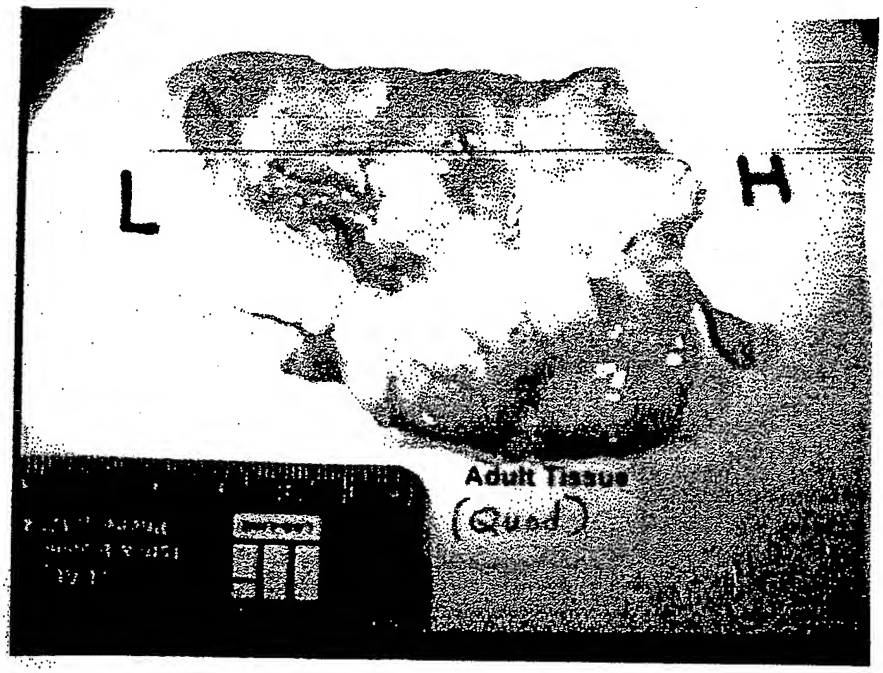

NE -11-035 Quadriceps tissue following injection of 200 mg/ml suspension of microsphere blanks (in colored vehicle)
L = normal 3% CMC vehicle ~ 53 cP
H = high viscosity suspension ([redacted] V4) ~ 730 cP
a mixture of 0.7 ml 3% CMC and 0.5 ml 10% CMC
A greater amount of separation of vehicle from the microsphere suspension was observed in the low vs the high viscosity vehicle suspension.
Injection sites were observed immediately following injection. In the high viscosity site the suspension was contained at the site until surgically opened, at which time it began to migrate across the entire tissue surface.

Work continued to Page

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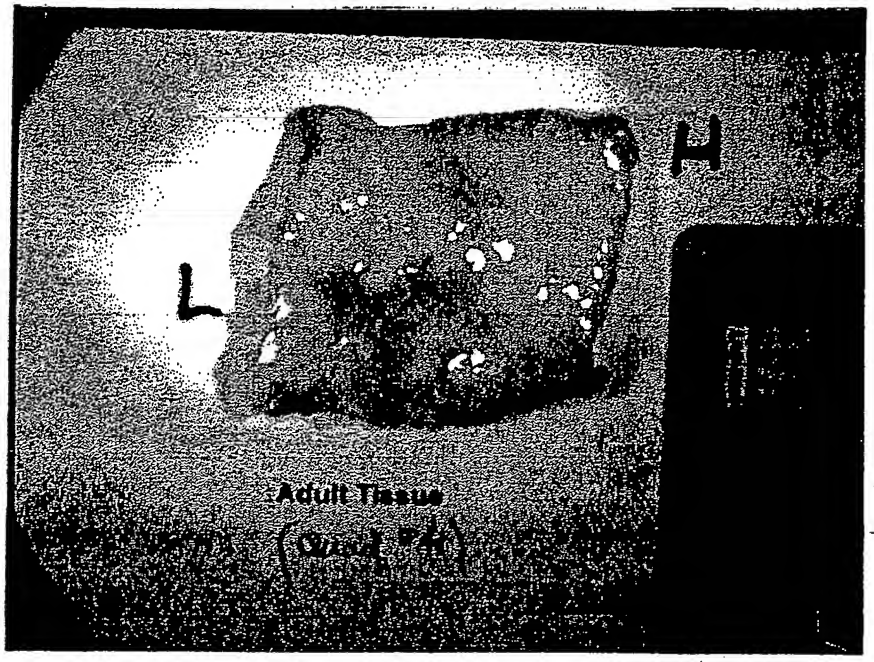

NE -11-041 Quadracept tissue following injection of 200 mg/ml suspension of black microspheres (in colored vehicle).
H = high viscosity vehicle ~730 cP (V4)
L = normal 3% CMC vehicle ~53 cP
The low viscosity suspension behaved exactly the same as in patent #035 ~~as~~ described on previous page.

Darrell Nye

Darrell Nye
Douglas Bissonnette

Investigation of the effects of viscosity on microsphere injectability

Study Objective: To determine if the injection failure rate is improved by increasing the viscosity of the vehicle microsphere mixture.

Methods: Blank microspheres (300 mg) will be suspended in an aliquot of 3% CMC vehicle. The microsphere suspension will then be mixed in a 3 cc syringe with increasing amounts of 10% CMC vehicle.

Dose (mg/mL)	3% CMC (mL)	10% CMC (mL)
1. 200*	1.2	0
2. 200*	0.9	0.3
3. 200*	0.7	0.5

* dose concentration assumes that the actual microspheres (300 mg) have a volume of 0.3 mL.

The viscosity will be measured using a viscometer and representative mixtures of 3% and 10% vehicle not containing microspheres.
Prior to autoclaving

refer to table #8 above

Vehicle Ratio (3%:10%)	%	RPM	Temp (°C)	Viscosity (cP)
1. 1.2:0.0	10.3	0.6	25.1	53.1
2. 1.1:0.1	12.4	0.3	"	121.6
3. 1.0:0.2	22.9	0.3	"	234.0
4. 0.9:0.3	61.4	0.3	"	628.5
5. 0.7:0.5				>1000

* Data collected

Autoclaved

Vehicle Ratio (3%:10%)	%	RPM	Temp (°C)	Viscosity (cP)
1. 1.2:0.0	14.6	0.6	25.0	74.6
2. 1.1:0.1	11.8	0.3	24.9	120.6
3. 1.0:0.2	17.5	0.3	25.1	180.9
4. 0.9:0.3	20.5	0.3	25.1	210.5
5. 0.7:0.5	92.4	0.3	25.1	945.2

* Data collected

Vehicle Ratio (3%:10%)	%	RPM	Temp (°C)	Viscosity (cP)
1. 1.2:0.0	10.4	0.6	25.0	53.1
2. 1.1:0.1	19.3	0.6	25.3	99.1
3. 1.0:0.2	18.3	0.3	25.3	187.0
4. 0.9:0.3	24.6	0.3	25.4	251.4
5. 0.7:0.5	71.8	0.3	25.4	730.6

* Data collected

Injectability of the blank microsphere suspensions will be evaluated using thawed porcine skin. Initially three replicates will be run of each viscosity to evaluate the effects of increasing viscosity on injectability, each injection will be rated as a success or failure. Injections will be made into the upper fat layer of the porcine skin using a 22 Ga TW needle.

Vehicle Ratio (3%:10%)	Injection 1	Injection 2	Injection 3
1. 1.2:0.0	-	+	-
2. 1.1:0.1	-	+	+
3. 1.0:0.2	+	+	+
4. 0.9:0.3	+	+	+
5. 0.7:0.5	+	+	+

* Data collected

Vehicle Ratio (3%:10%)	Injection 1	Injection 2	Injection 3
1. 1.2:0.0	+	+	-
2. 1.1:0.1	+	-	+
3. 1.0:0.2	+	+	+
4. 0.9:0.3	+	+	+
5. 0.7:0.5	+	+	+

* Data collected

Injectability curves: A series of injectability curves will be generated for the grids below using 21, 22, and 23 Ga TW needles. Injectability will be determined using a 5x5" piece of pig flesh which has been stored at -20°C. The tissue will be thawed, and maintained at room temperature in normal saline (0.9% NaCl). Microspheres will be suspended in 0.9 mL 3% CMC vehicle, 0.9 mL of the suspension is withdrawn from vial and combined with varying amounts of a 3/10% mixture to obtain the final vehicle viscosity.

21 Ga needle

Ratio (3:10%)												
1. 1.2:0												
2. 1.1:0.1												
3. 1.0:0.2												
4. 0.9:0.3												
5. 0.7:0.5												
µs conc (mg/mL)	175			200			250			300		

22 Ga needle

Ratio (3:10%)												
1. 1.2:0	-	+	+	+	-	-	-	-	-			
2. 1.1:0.1	-	-	+	-	+	+	+	+	+			
3. 1.0:0.2	+	+	+	+	+	-	+	-	-			
4. 0.9:0.3	+	+	0	+	+	+	+	+	+			
5. 0.7:0.5												
µs conc (mg/mL)	175			200			250			300		

* Data acquired

22 Ga needle

Ratio (3:10%)												
1. 1.2:0												
2. 1.1:0.1												
3. 1.0:0.2												
4. 0.9:0.3												
5. 0.7:0.5	X	X	X	X	X	X	X	X	X	X	X	X
µs conc (mg/mL)	150			190			250					

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Injectability of Risperidone Microspheres in Pigs: Effect of Particle Size, Injection Vehicle Viscosity and Injection Site

SUMMARY

The injectability of risperidone microspheres was evaluated in Yorkshire weanling pigs (Study AT-10-01). The objectives of the study were (1) to evaluate the utility of the weanling pig as an animal model for assessment of IM injectability of microspheres, (2) to identify critical parameters affecting injectability, and (3) to provide animal data on the effects of microsphere particle size on injectability.

It was found that the conditions that most closely mimic the current clinical protocol for administration (160 mg microspheres per mL vehicle; the current clinical vehicle; injection in hind quarters) did not provide a readily measurable injection failure rate (0 failures out of 10 injections). Higher failure rates were observed when a less viscous injection vehicle was employed or when microspheres were injected into a smaller muscle (i.e., the leg).

The effect of microsphere particle size on injectability was evaluated under two conditions: (1) less viscous vehicle in the hind quarter and (2) current clinical vehicle in the leg. These conditions were selected in order to provide a measurable failure rate with the current particle size distribution (<180 µm). A particle size dependence was observed under both conditions; fewer failures occurred with the <125 µm and <150 µm preparations compared to the <180 µm material. The <125 µm and <150 µm preparations were indistinguishable with respect to the frequency of injection failure.

The data afford the following conclusions:

- The pig may provide a useful model for evaluation of risperidone microsphere injectability. However, the low failure rate observed under conditions intended to simulate current clinical practice makes it necessary to perform experiments under conditions that increase the likelihood of injection failure in order observe improvements in injectability while avoiding excessive expenditure of time and materials. Defining these conditions will require additional model development work.

- IM injectability of risperidone microspheres is dependent on injection vehicle viscosity and microsphere particle size, and to a lesser extent on the site of injection and the concentration of the microsphere suspension. Reducing the injection vehicle viscosity led to a higher rate of injection failures due to needle clogging. Lower failure rates were observed with microspheres that had been fractionated to remove particles greater than 150 µm in diameter.

MATERIALS

Test articles are listed in Table 1. Risperidone microspheres were manufactured at the 125 gram scale in the Wilmington facility. Microspheres were sized to <125 µm and <150 µm using USA Standard Testing Sieves Nos. 120 and 100, respectively.

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The current clinical injection vehicle (1.5% CMC, 30% sorbitol, 0.2% Tween 20) was provided by Janssen Pharmaceutica (Lot 23839). The lower viscosity vehicle (0.75% CMC, 15% sorbitol, 0.2% Tween 20) was prepared at the Blue Ash facility (Lot 96-13-103).

EQUIPMENT

19G TW x 1.5 inch hypodermic needles (B-D Precisionglide® cat. no. 305187)
3 cc hypodermic syringes (B-D cat. no. 309585)

METHODS

Animal studies were performed at Charles River Pharmservices, Inc. (Southbridge, MA). Study outlines are attached.

Injection experiments were conducted in male and female Yorkshire weanling pigs approximately 6 weeks in age (10-15 kg). Animals were anaesthetized with low doses of Telazole and Xylazine and with halothane if needed. Injection sites were shaved, cleansed and with betadine swabs prior to microsphere administration. Injections to the hind quarters were administered to the biceps femoris in the upper hind limb. Injection sites in the legs were as follows: Forelimb—superficial digital flexor muscles; hindlimb—cranial tibial muscle.

Microspheres and injection vehicle were equilibrated to ambient temperature for at least 30 minutes. Using a 3 mL syringe equipped with a 1 1/2 inch 19 gauge thin wall needle, the prescribed volume of injection vehicle was withdrawn into the syringe and injected into the vial containing microspheres. The microspheres were suspended in the vehicle by orienting the vial horizontally and rolling it between the palms of the operators hands. This was done without removing the needle/syringe from the septum. The time required to fully suspend the microspheres was approximately one minute.

The suspended microspheres were then withdrawn into the same needle/syringe and injected. Following insertion of the needle and prior to injection of the suspension, the syringe plunger was withdrawn slightly to confirm that the needle was located in the extravascular space. The time interval between aspiration of the suspension and injection was usually less than one minute.

Animals were sacrificed within approximately 24 hours following dosing. Injection regions were evaluated to pinpoint the site of microsphere deposition and to assess the distribution of microspheres in the tissue.

RESULTS

Preliminary Study: An initial range finding study was performed in order to assess the rate of injection failures under conditions approximating the current clinical practice (particle size <180 µm, current vehicle, injection in hind quarters, 160 mg microspheres per mL vehicle) and to evaluate the effects of (1) decreased injection vehicle viscosity, (2) injection in a less compliant site and (3) a twofold increase in microsphere concentration. Results are summarized in Table 2.

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No failures occurred in 10 injections performed using the clinical procedure (Table 2). Increased failure rates were observed with the lower viscosity vehicle (4 failures per 7 injections) and when microspheres were injected in the leg (1 failure per 8 injections). No failures were observed in four trials performed at the higher microsphere concentration.

Particle size effects: Table 3 summarizes injectability data for microspheres fractionated by size. Similar trends were observed when the system was stressed either by decreasing the vehicle viscosity or by injecting in the leg. In both cases, failure rates were higher with the <180 μm fraction. The <125 and <150 μm fractions were indistinguishable in terms of failure rate.

An effect of microsphere concentration was observed in the injections where <180 μm microspheres in the higher viscosity vehicle were administered to the leg (Table 3).

Injection site observations: Following sacrifice, injection sites in the hind quarters were examined in order to determine the location of microsphere deposition and the spatial distribution of the injected microspheres. It was observed that in most cases, microspheres were deposited intramuscularly and showed a focal and linear distribution in the tissue. In a few cases, microspheres were deposited intermuscularly and exhibited a focal distribution.

DISCUSSION

Pig model utility: The 0% failure rate observed under current clinical conditions (Table 2) is not unexpected in view of the low failure rate observed in human patients (<5% as reported by Janssen). This suggests that the pig study procedure is not appreciably more prone to failure than administration of microspheres to human patients. Therefore, in order to detect improvements in injectability stemming from changes in formulation or methodology (e.g., reduction in maximum particle size), it is necessary either to increase the number of injections substantially or to modify the experimental conditions in order to make the system more discriminating. The former option is potentially costly in terms of time and material and in this case was not possible due to the limited quantity of microspheres available. Modifying the conditions, on the other hand, should provide useful data as long as the process leading to injection failure does not change as a result of the modification.

Based on the results in Table 2, the vehicle viscosity and the site of injection were selected as parameters to modify in order to provide conditions suitable for assessing the effects of microsphere particle size on injectability. Because the particle size effects were similar in both cases, it is unlikely that the effects are an artifact of the modified conditions.

Additional model development work will be required in order to define appropriate conditions for a given experimental objective. For example, a study aimed at optimizing injection vehicle viscosity would require modification of a parameter other than vehicle viscosity in order to ensure a measurable failure rate (e.g., site of injection).

Risperidone microsphere injectability: These studies indicate that microsphere injectability is strongly dependent on microsphere particle size and injection vehicle viscosity and somewhat dependent on the site of injection and the concentration of the microsphere suspension. The particle size effects observed are qualitatively consistent with in vitro results obtained by the Development group at Blue Ash (described in

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Development Report dated . On the other hand, the in vitro data do not predict the viscosity dependence observed in the animal studies.

REFERENCES

AT-10-01 Study Outlines (attached)

Notebook references:

Injectability study: Notebook -62 pp. 1-24.
Injection site characterization: Notebook -62 pp. 58-59.

Reports:

In vitro injectability of Risperidone microspheres (Development Report dated

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Table 1. Test articles

Study	Description	Vial fill	Lot number
Preliminary range finding			
Phase 1, 2a, 2b	<180 µm	160 mg	136-2196[1]
Phase 3	<180 µm	320 mg	136-2966[2]
Effect of particle size			
	<180 µm	320 mg	136-2966[3]
	<150 µm	320 mg	136-2966[3]
	<125 µm	320 mg	136-2966[3]

[1]Designated as ALK961119a in study outline
[2]Designated as ALK961119b in study outline
[3]Designated as ALK961122 in study outline

Table 2. Effect of injection vehicle, injection site and microsphere concentration on injectability[1]

Phase of study	Vehicle viscosity	Microsphere dose	Volume	Site	Failure rate
1	High[2]	160 mg	1 mL	Hind quarter	0/10
2a	High	160 mg	1 mL	Leg	1/8
2b	Low[3]	160 mg	1 mL	Hind quarter	4/7
3	High	320 mg	1 mL	Hind quarter	0/10

[1]Microsphere particle size <180 µm
[2]Current clinical vehicle: 1.5% CMC, 30% sorbitol, 0.2% Tween 20
[3]0.75% CMC, 15% sorbitol, 0.2% Tween 20

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Table 3. Effect of particle size on injectability

Max. particle size (μm)	Vehicle viscosity	Volume (mL)	Site	Failure rate	Avg. % delivered (failed injections)[1]
180	High[2]	2.0	Leg	0/5	n/a
150	High	2.0	Leg	0/5	n/a
125	High	2.0	Leg	0/5	n/a
180	High	1.0	Leg	2/4	0
150	High	1.0	Leg	0/4	n/a
125	High	1.0	Leg	0/4	n/a
180	Low[3]	2.0	Hind quarter	8/10	33
150	Low	2.0	Hind quarter	2/10	18
125	Low	2.0	Hind quarter	3/10	80

[1]Average fraction of dose delivered prior to needle clog (failed injections only)
[2]Current clinical vehicle: 1.5% CMC, 30% sorbitol, 0.2% Tween 20
[3]0.75% CMC, 15% sorbitol, 0.2% Tween 20

ALKERMES, INC

DEVELOPMENT REPORT

Title:	EFFECT OF VEHICLE VISCOSITY ON INJECTABILITY OF RISPERDAL® DEPOT
Prepared By:	J. Michael Ramstack Norman N. Kim
Date:	
Product:	RISPERDAL® Depot

SUMMARY

Intramuscular injectability tests were conducted in sheep inorder to identify a minimum specification for vehicle viscosity to ensure successful injection of the RISPERDAL® Depot suspension in the clinic. Under "stressed" conditions, i.e. significantly smaller needle gauge and higher suspension concentration than expected in the clinic, vehicle viscosities ≥ 23cp were found necessary for successful injections. Viscosities of ≤ 11cp produced a high level of failures. Data also indicates vehicle density positively influences injectability (presumably following Stokes Law).

INTRODUCTION

The results from an earlier investigation[1] identified vehicle viscosity as a key variable affecting injectability. As part of a program to revise vehicle formulation, additional data is required in order to establish a useful minimum viscosity specification. The objective of these studies is to characterize the effect of vehicle viscosity on the *in vivo* intramuscular injectability of RISPERDAL® Depot and to recommend a minimum viscosity specification.

MATERIALS

RISPERDAL® Depot microspheres (Batch 1Kg-0908-7) were sieved through a 150μ screen and filled in microsphere doses of 150 or 300mg into 5cc siliconized vials (Schott) and sealed with Teflon faced septum (West). The packaging materials are representative of the Phase III clinical trial product.

ProLease placebo microspheres (RG502H) were sieved through a 106 μ screen and filled in a microsphere dose of 300mg in serum/lyo flint vials (West) and sealed with gray Purcoat V-32 septum (West).

A number of vehicle formulations were prepared of various compositions. These formulations are based on mixtures of three basic starting formulations listed in Table 1. The test vehicles are listed in Tables 2 and 3: Viscosities were determined by Brookfield Model LVT viscometer fitted with UL adapter.

Animal injectability tests were conducted using 3cc syringes (B-D) and 22G TW x 1.5 inch needles(B-D) exclusively.

Table 1: Starting Vehicles

Starting Vehicle	Lot No.	Composition
Janssen Vehicle	130/1-65	1.5% carboxymethylcellulose (CMC), 30% sorbitol, and 0.2% Tween20
ProLease Diluent	0316	3% CMC, 0.1% Tween 20, and 0.9% NaCl
Saline Vehicle	0315	0.9% NaCl, 0.1% Tween 20

METHODS

Animal studies were conducted at Charles River Pharmservices (Southbridge, MA) using domestic sheep weighing approximately 100-150 lbs. Although earlier studies were conducted with pigs, the sheep was found a more preferred model for intramuscular injectability. Based on ultrasound and gross observation, sheep possess a greater surface area of uniform muscle thickness with a low fat content.

Animals were anesthetized with Telazole/Xylazine/Atropine intramuscularly and further supplemented with isofluorane gas (~1-2%) during the injection procedure. Prior to injection, the animal's dorsal, gluteal, and upper leg regions were shaved and cleaned with alcohol. Injection sites were visualized prior to and during dosing using ultrasound (EI Medical).

Microspheres and vehicle were equilibrated to ambient temperature prior to dose suspension. Using a 3 cc syringe and 22 gauge thin-walled needle, vehicle was aspirated and injected into the microsphere vial. RISPERDAL Depot microspheres were suspended in 1 mL of vehicle at approximate concentrations of 130 or 230mg/mL (~1.15 or 1.3mL total volume). Placebo microspheres were suspended in 2 or 1 mL of vehicle at approximate concentrations of 130 or 230 mg/mL (~2.3 or 1.3 mL total volume). The vial was then agitated by hand for approximately 1 minute until microspheres were suspended. The suspension was then aspirated back into the syringe using the same needle. Care was taken to recover the maximum amount of suspension from the vial. Preparation of dose suspensions were conducted randomly by three individuals.

All doses were injected by a single individual into the animal almost immediately after preparation. The rate of injection was maintained constant at approximately 5-10 sec. Following the injection procedure, animals were euthanized by an overdose of sodium pentobarbital.

RESULTS AND DISCUSSION

The study was composed of two parts. In Part I (Phases 1 and 2) both RISPERDAL Depot and ProLease placebo microspheres were studied at two suspension concentrations of 130 and 230 mg/mL using the starting saline, Janssen or ProLease vehicles. Additional tests were conducted (Phase 3) with dilutions of the Janssen vehicle with saline. The results are reported in Table 2.

Table 2: Results of Part 1

Phase	Treatment	Concentration (mg/mL)	Vehicle	Viscosity (cp)	Failures/ Injections
1	RISPERDAL Depot	130	Saline	1	8/10
	"	"	Janssen	24	1/10
	"	"	ProLease	56	0/10
	ProLease Placebo	"	ProLease	56	1/10
2	RISPERDAL Depot	230	Janssen	24	0/10
	"	"	ProLease	56	0/10
3	RISPERDAL Depot	230	3:1 Janssen:Saline	11	0/5
	"	"	1:3 Janssen:Saline	2	7/10

In Part I, the Phase 3 vehicles were prepared by diluting Janssen vehicle with saline vehicle. This dilution represented not only changes in viscosity due to CMC dilution, but also changes in density due to sorbitol dilution. In order to study the effect of viscosity alone, additional tests (Part II) were conducted using vehicles prepared by diluting only ProLease vehicle with the saline vehicle. The results are listed in Table 3.

Table 3: Results from Part II

Phase	Treatment	Concentration (mg/mL)	Vehicle	Viscosity (cp)	Failures/ Injections
1	RISPERDAL Depot	230	Saline	1	10/10
	"	"	Diluent 3 *(1:1 Diluent 2:Saline)*	1	8/10
	"	"	Diluent 2 *(1:1 Diluent 1:Saline)*	11	5/10
	"	"	Diluent 1 *(1:1 ProLease:Saline)*	23	1/10
	"	"	ProLease	64	2/10
	ProLease Placebo	130	ProLease	64	0/10
2	RISPERDAL Depot	230	Diluent 4 *(1:1 ProLease:Diluent 1)*	38[1]	2/10
	ProLease Placebo	"	Diluent 4	38[1]	0/5

[1] Estimate. Insufficient sample for measurement.

The results of the ProLease/Saline vehicle combinations in Part I and II show that vehicle viscosity has a clear effect on injectability. Based on the data, it appears viscosities at least above ~23 cp are necessary for successful injection of RISPERDAL Depot. At ~11cp or less, injection failures increase significantly.

Comparing the 11cp data in both Part I and II indicates that solution density may also play a role in affecting injectability outcome. The 3:1 dilution of Janssen vehicle with saline in Part I resulted in a viscosity of ~11cp and no injection failures. Calculated sorbitol content (22.5%) of this sample, however is appreciable. Sorbitol is added to retard microsphere settling by adjusting the fluid density to better match the microspheres. Compared to this diluent in Part 1, the ~11cp sample in Part II, Dilution 2 with no sorbitol, resulted in 5/10 failures.

Injectability failure is thought to be due to microspheres separating from the mainstream of vehicle flow, accumulating and eventually causing a plug. The action of both fluid density and viscosity on injectability is consistent with Stokes Law which may, in part govern the separation process.

Suspension concentrations of 130 and eventually 230mg/mL and needle gauges of 22 TW were used in this study. In clinical practice, the maximum suspension concentration of ~100mg/mL (75mg active drug in 2mL vehicle) and needle gauge of 20 UTW is proposed for RISPERDAL Depot. Higher concentrations and smaller needle gauges were used in this study inorder to "stress" the system and provide positive workable data.

The RISPERDAL Depot microspheres suspended more readily and displayed less vial adhesion and hold-up compared to the ProLease Placebo microspheres. This is may be due to the vials containing RISPERDAL Depot were siliconized.

NOTEBOOK REFERENCES:
NB -16, pgs 65-66, 71

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[1] Alkermes Product Development Report, Injectability of Risperidone Microspheres in Pigs: Effect of Particle Size, Injection Vehicle Viscosity, and Injection Site, AT-10-01, issued